Faculty of Resource Science and Technology

Germination of *Hibiscus sabdariffa* L. following Priming Treatments

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DECLARATION

I hereby declare that no portion of this project work has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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List of Abbreviation

PEG	Polyethylene glycol
KOH	Potassium hydroxide
CRD	Completely Randomized Design
ANOVA	Analysis of Variance
HSD	Honestly Significant Difference

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ABSTRACT

A study was conducted to evaluate the effectiveness of priming treatments in improving the germination of *H. sabdariffa* seeds. Three types of treatment used were hydropriming, osmopriming, and thermopriming. The seeds were scarified using sand paper to enhance imbibitions of water. Priming agents included distilled water, PEG 8000, and warm water at 40^0C, were used to prime the seeds for period of 0, 6, 12, 18, and 24 hours and evaluated for moisture content and germination. Results showed that highest moisture contents were from scarified *H. sabdariffa* seeds which were 74.31% in hydropriming, 72.21% in osmopriming (0.1 g/ml H_2O PEG 8000), and 81.99% in thermopriming. Scarified seeds thermoprimed at 40^0C gave the highest germination percentage than other treatments that was 82% at 18 hours of priming. Generally, germination was increased and more uniform in all types of priming which indicated that seeds were exposed to favourable condition and achieved suitable physiological state for seed germination.

Key words: *H. sabdariffa* seeds, priming treatments, scarification, moisture content, germination

ABSTRAK

Satu kajian telah dijalankan bagi menilai keberkesanan rawatan 'priming' dalam meningkatkan kadar percambahan dalam biji benih H. sabdariffa. Kajian yang telah dilakukan terdiri daripada tiga jenis rawatan iaitu 'hydropriming', 'osmopriming', and 'thermopriming'. Biji benih H. sabdariffa telah diskarifikasikan menggunakan kertas pasir untuk meningkatkan kadar kemasukan air. Agen 'priming' yang berlainan iaitu air suling, PEG 8000, dan air panas bersuhu 40^0C telah digunakan untuk rawatan yang berlainan bagi tujuan merendamkan biji benih selama 0, 6, 12, 18, dan 24 jam dan digunakan untuk menentukan kandungan kelembapan dan percambahan. Hasil kajian menunjukkan kandungan lembapan tertinggi adalah pada biji benih H. sabdariffa yang telah diskarifikasikan iaitu 74.31% dalam 'hydropriming', 72.21% dalam 'osmopriming' (0.1 g/ml H_2O PEG 8000), dan 81.99% dalam 'thermopriming'. Biji benih yang telah diskarifikasikan dan direndam dalam air bersuhu 40^0C menunjukkan peratus percambahan tertinggi berbanding rawatan 'priming' yang lain, iaitu 82% pada jangka masa 18 jam. Secara umumnya, peratusan percambahan biji-biji benih telah meningkat dan seragam dalam kesemua rawatan 'priming' dan ini menunjukkan biji-biji benih telah terdedah kepada keadaaan yang sesuai dan mencapai keadaan fisiologikal yang baik untuk percambahan.

Kata kunci: Biji benih H. sabdariffa, rawatan 'priming', skarifikasi,kelembapan, percambahan

1.0 INTRODUCTION

Hibiscus sabdariffa L. or commonly known as Roselle belongs to family Malvaceae. It is a short-day annual plant that closely resembles cranberry (*Vaccinium* spp.) in flavor (Morton, 1987). Roselle is known as asam susur in Malaysia, Gamet Walanda and kasturi roriha in Indonesia, krachiap-daeng, krachiap-prieo and phakkengkheng in Thailand and kubab and talingisag in Philiphines (Dasuki, 2001). Vernacular names for *H. sabdariffa* are sorrel, rozelle, red sorrel, Jamaica sorrel, India sorrel, Guinea sorrel, sour-sour, Queensland jelly plant, jelly okra, lemon bush and Florida cranberry (Morton, 1987). It is native of Africa, but some do believed that it originated from India (Dasuki, 2001). Nowdays *H. sabdariffa,* is widely distributed in the tropics and subtropics of both hemispheres, and in many areas of West Indies and Central America (Morton, 1987).

H. sabdariffa is an erect annual herb, 0.5-3 m tall, stem glabrous, purplish; leaves broadly ovate-orbicular, variable, 6-15 cm long, lobes 3-5, oblong to lanceolate, glabrous or pubescent, purplish, petiole 5-10 cm long; bracteoles of epicalyx 8-12, calyx after anthesis becoming thick-fleshy, 2.5-5.5 cm long, distinctly longer than the fruit, red, corolla 3-5 cm long, not widely open, pinkish to yellow with purple centre; seed reniform, almost glabrous, black brown (Dasuki, 2001).

The “flower buds” are actually seedpods enclosed in their fleshy calyxes. The red pods or fruits and calyxes are fused and difficult to distinguish from one another (Apell, 2003). Roselle grows well on sandy loam and tolerant to drought. *H. sabdariffa* is propagated solely by seed. Cultivation of roselle using cuttings produce short plants and yield of calyxes is relatively low (Morton, 1987).

H. sabdariffa is attacked mainly by root nematodes (Morton, 1987) and Phytophtora parasitica mainly in early stage of its development in the field (Follin, 1996). Alegbejo *et al*., (2003) stated that *H. sabdariffa* has a lot of potential as an industrial crop. Smallholder and resource poor farmers grow it. It is popularly grown for its fiber content and for its leaves, calyxes, roots and seeds, which are used either for industrial, medical, commercial or domestic uses.

H. sabdariffa and its parts can be utilized for many purposes (Duke, 1983). It is used as health product, yielding fiber, beverage, edible foliage, and an oil seed. *H. sabdariffa* is attracting the attention of food and beverage manufacturers and pharmaceutical companies who feel it may have possibilities as natural food product and as a color to replace some synthetic dyes (Morton, 1987).

H. sabdariffa is among the plant resources that are rich in calcium, niacin, riboflavin and iron. In India, Africa and Mexico, all the above ground parts of the *H. sabdariffa* plant are valued in native medicine (Morton, 1987). The local infuse the leaves or calyxes to treat diuretic, choleretic, febrifugal and hypotensive decreasing the viscosity of blood and stimulating intestinal peristalsis. Some persons used it to relieve colds and heated leaves to heal scars and wounds. Heated leaves also applied to cracks in the feet and on boils and ulcers to speed maturation. A lotion made from *H. sabdariffa* leaves is used on sores and wounds.

Duke (1983) reported that *H. sabdariffa* be antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, refrigerant, resolvent, sedative, stomachic, and tonic. It is also a folk remedy for abscesses, bilious conditions, cancer, cough, debility, dyspepsia, dysuria, fever, hangover, heart ailments, hypertension, neurosis, scurvy, and strangury. The drink made by placing, the calyx in water, is said to be a folk remedy for cancer. Perry (1980) cites one study showing *H. sabdariffa* usefulness in arteriosclerosis and as an intestinal antiseptic.

Utilization of this plant is varied among countries. In Burma the seed are used for debility and the leaves as emollient. Taiwanese regard the seed as diuretic, laxative, and tonic. Philiphines use the bitter root as an aperitive and tonic (Perry, 1980). Angolans use the mucilaginous leaves as an emollient and as a soothing cough remedy. Central Africans poultice the leaves on abscesses. Besides that, simulated ingestion of the *H. sabdariffa* extract decreased the rate of absorption of alcohol, lessening the intensity of alcohol effects in chickens (Watt and Breyer- Brandwijk, 1962). This means *H. sabdariffa* can help to maintain human body from the cardiovascular diseases in which cholesterol plays a major role. It is done by reducing fats in the body, suitable for people under diet programme and weight reduction. Tee *et al.*, (2002) agreed that roselle might play a role in the prevention of atherosclerosis and obesity.

Other than has higher potential in medicinal aspect, this species also encouraging in food industry. According to Watt and Breyer- Brandwijk (1962), *H. sabdariffa* fruits are edible. Fresh calyxes can be consumed immediately by added into fruit salads. Calyxes are stewed into sauce, syrup, marmalade, relish and chutney (Morton, 1987). *H. sabdariffa* syrup or sauce may be added to puddings, cake, frosting and salad dressings. It can also be poured over gingerbread, pancakes, waffles or ice cream.

This species possesses 3.19% pectin and has been recommended as a source of pectin for fruit preserving industry (Morton, 1987). The efficacy of roselle as a functional food has been revealed lately, especially for its antioxidant bioactivity (Tee *et al*., 2002). Roselle leaves are sometimes eaten raw but are mostly cooked with Egusi (*Cucmumeropsis edulis*) to make a pleasant sour tasting source, usually served with tufu.

Seeds of *H. sabdariffa* have become an important source of planting material which is fundamental to agricultural industry. Currently, *H. sabdariffa* is propagated by seeds. The germination of *H. sabdariffa* depends on variety. Thus, the production of high quality seeds of *H. sabdariffa* are needed for gaining the good and high quality produces. In this study, the seed germination of *H. sabdariffa* have been accessed by three different type of priming treatments based on several number of priming agents.

The main objectives of this study are to assess the germination of *H. sabdariffa* seeds used as planting material, to evaluate the influence of priming agents on the germination of *H. sabdariffa* seeds and to determine the most effective priming treatments in improving the germination of *H. sabdariffa* seeds.

2.0 LITERATURE REVIEW

2.1 Seed Moisture Content

The moisture content is the amount of the water in the seed usually expressed in percentage. It can be expressed either a wet-weight basis, where it is expressed as a percentage of the fresh weight of the seed or on a dry weight basis, where it is expressed as a percentage of the dry weight of the seed. It will give effect to the storage life of the seeds if there any small change in the seed moisture content. Hanson (1985) reported that is important to know the moisture content in order to make a reasonably accurate prediction of the possible storage life of each accession.

When moisture content is higher than 30%, the non-dormant seeds may germinate, and from 18% - 30% moisture content rapid deterioration by microorganisms can occur (Bewley and Black, 1995). Seed stored at moisture content that higher than 18% - 20% will respire, and in poor ventilation that generated heat will kill them. For the seeds that are below than 8% - 9% moisture content, there is little or no insect activity, and for the below than 4% - 5% moisture content seeds are immune from attack by insect and storage fungi, they may deteriorate faster than those maintained at slightly higher moisture content.

Seed have to be stored at a critical moisture content which is usually little lower than the seed moisture content at harvest and lost viability with decrease in their moisture content (Mittal *et al*., 1998). At critical moisture content, seeds are physiologically matures and are at optimum and maximum viability, germination and storage.

2.2 Seed Viability

The definition of viability is the ability of a seed to germinate and produce a “normal” seedling (Copeland and McDonald, 1995). Viability also indicates the degree to which a seed is alive, metabolically active, and possesses enzymes capable of catalyzing metabolic reactions needed for germination and seedling growth. Seed viability is probably highest at the time of physiological maturity through environment conditions on the parent plant may not permit germination. After physiological maturity, the viability of seeds gradually declines. Their longevity depends on the environmental conditions to which they are exposed. Viability needs to be determined at the start of storage and at regular intervals during storage to predict the correct time for regeneration of the accession (Hanson, 1985). The viability test takes from a few days to weeks or even months to give an accurate result.

There are numerous test exist for determining seed viability. The tetrazolium (TZ) test is widely recognized as an accurate means of estimating seed viability (Copeland and McDonald, 1995) and this test is very useful for rapidly obtaining an indication of germination potential and viability of samples and is in extensive use (Agrawal, 1980). The advantage of TZ test is the rapidly of the test and may be justified its use when speed is important. Other than that, it also can be useful for testing dormant seed when it is used in combination with a germination test. The germination test tells the percentage of immediate germination while the TZ test tells the percentage of live seeds. The difference between these two tests represents the percentage of dormant seed (Copeland and McDonald, 1995).

2.3 Seed Germination

According to Fenner and Thompson (2005), germination is a process that involves the imbibitions of water, a rapid increase in respiratory activity, the mobilization of nutrient reserves and the initiation of growth in the embryo. This process is irreversible which once germination has started; the embryo is committed permenantly to growth or death. The seed physiologist defined germination as the emergence of the radicle through the seed coat. As for the seed analyst, germination is "the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favorable conditions" (AOSA, 1991). Germination test is conducted to estimate the maximum number of seeds that can be germinated in optimum condition. The high quality seeds are germinating maximum at physiologically maturity. Otherwise, deterioration will occur due to ageing, effect of environmental conditions, and any damage sustained during collection, processing and storage.

2.4 Seed Storage

The purpose of seed storage is to preserve planting stocks from one season to the next and also to maintain the seed quality for the longest duration possible. This will create a greater diversity in seed inventory and provides a guarantee of seed supply in years when acceptable seed quality and production is low (Copeland and McDonald, 1995). The maintenance of high speed germination and vigor from harvest until planting is very important (Agrawal, 1980). Seeds are practically worthless if upon planting, they fail to give adequate plant stands. Therefore, good seed storage is a basic requirement in seed production. According to Ells *et al*., (2009), conditions essential to good seed storage are just the opposite of those required for good germination. Good germination occurs when water and oxygen are present at a favorable temperature. Good seed storage results when seeds are kept dry, below 8 percent temperature is kept low and below 40 degrees.

2.5 Seed Dormancy

Leadem (1997) stated that many plants have dormant seeds as a biological mechanism to ensure that seed will germinate at a time and under conditions that are favorable for the growth and survival of the next generations. There are two types of dormancy which are exogenous (seed coat dormancy) and endogenous (embryo dormancy). Some species seeds have hard seed coats preventing imbibitions of water and the exchanges of gases. Without imbibitions and gas exchange, germination would be impossible. Physical seed coat dormancy occurs frequently in species adapted to alternating dry and wet seasons, including leguminous genera (Willan, 1985). There are several treatments to break dormancy for the purpose of inducing germination.

2.6 Seed Scarification

Seed coat that makes the external dormancy is results from a seed's hard seed coat that is impervious to water and gases. The seed will not germinate until the seed coat is altered physically. Seed scarification is any process of breaking, scratching, or mechanically altering the seed coat to make it permeable to water and gases. In nature, scarification often occurs by the seed falling. Freezing temperatures or microbial activities modify the seed coat during the winter. Seeds are also scarified as they pass through the digestive tract of animal (Blazich & Evans, 1999).

Scarification can be forced, rather than waiting for nature to alter the seed coats. Scarification involves a process to overcome physical seed coat dormancy, is designed to soften, puncture, scratch, or split the seed coat in order to make seed permeable to water and gases without damaging the embryo and endosperm within the seed. Seed scarification is consists of physical and biological method, dry heating, or soaking in water or chemical solutions (Willan, 1985).

2.6.1 Physical method

One of the simplest and most direct physical methods is to cut, drill or file a small hole in the seed coat before sowing. This method has been found to be successful in many legumes. Sand paper may also be used to reduce seed coat thickness by abrasion, especially on hard-coated species. Manual treatment of individual seeds is slow but can be safe and effective. This process is best suited for larger seeds. Soaking in cold water before sowing reinforces physical scarification (Willan, 1985).

2.6.2 Soaking

A number of treatments involve soaking seeds in water or other liquids. These treatments may soften hard seed coats or leach out chemical inhibitors. Hot water treatment has yielded beneficial results with a number of leguminous seeds. The seeds are usually placed into boiling water, immediately removed from the heat source, and left to cool gradually while the seeds remaining in the water for about 12 hours. Soaking in boiling water makes the seed coats permeable to water (Phartyal *et al.*, 2005). They imbibe and swell as the water cools. It has been shown that the initial water temperature has a bigger effect on germination rate than the periods of soaking and cooling of the seed (Willan, 1985). This treatment is recommended for species that have little resistance to germination. Prescription for hot water must be applied carefully without killing the seeds through seed excessive heating.

2.6.3 Acid treatment

Acid treatments mimic part of biological method of seed scarification. Seed coats can be effectively broken down by chemical means whether that is stomach acid or concentrated sulphuric acid, the chemical most commonly used to break seed coat dormancy. Seeds that have been dormant for a long period may require longer soaking periods in the acid than fresh seeds. Due to toughness of the seed coat varies between species and within species, the optimum period of immersion in acid for each lot may be treating a small sample for different periods and the soaking the lots in water at room temperature for 1 to 5 days.

Over soaking may pit the seed and even expose the endosperm. Insufficient soaking leaves the seed coat of most species glossy. Coats of correctly treated seeds are dull and not deeply pitted. Sulphuric acid treatment has been effective for several temperate and subtropical species, for example *Gleditsia triacanthos* (Willan, 1985).

2.6.4 Biological method

In nature, the frost, rain action and soil microbes carry out natural scarification. Animal and microorganisms are important factors in breakdown of seed coat impermeability. Seed coats are broken down as they pass through the digestive system track of animals because of the action of the strong digestive chemicals. Termites are important agent for breaking down seed coat dormancy in many parts of the tropics. This treatment is efficient for certain tropical fruit like teak fruit (*Pterocarpus angolensis*). Seeds are spread on the ground in a 5 cm thick layer immeadiately after collection and covered with cardboard. After five weeks, the termites will remove the exocarp, improving germination rates (Willan, 1985). Partial fermentation, which could be damaging many seeds, is beneficial to others to overcome seed coat dormancy. Fruit of *Tectona grandis* are laid on the ground through the rainy season and then collected and stratified (Willan, 1985).

2.6.5 Dry heat and fire

In the seasonal wet and dry tropics, fire is powerful natural factor in the removal of seed coat dormancy. Fire treatment has been implemented successfully for seeds of species such as *Tectona* and *Aleurites moluccana*. The seeds are spread evenly on the ground and then covered with grass, which set on fire. As soon as the grass in burned, the seeds are placed in cold water. The quick temperature change causes the seeds to crack open (Willan, 1985). The dry heat cracks the seed coat, which accelearates imbibition (Schelin *et al.*, 2004). Seeds that experience too high temperatures or high temperatures that last too long germinate less frequently. For this reason, care needs to be taken when dry heating is used as treatment method.

2.7 Seed Priming

Priming is a process applied on seed to strengthening or increase vitality of seed whereas hydration is known as process of supplying water to seed to maintain the fluid or moisture content in seed. Germination rates of seed can be accelerated through the process of priming where this treatment would improves the seed vigor as it produce uniform seedling with faster germination rates (Walters, 1998).